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# Microbial communities and enzymatic activities under different management in semiarid soils<sup>☆</sup>

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## ABSTRACT

Information about the size, composition and ecological role of soil microorganisms remains unknown for some semiarid regions of the world while soil functioning and productivity depend on its biological component. This study evaluated the microbial communities and enzyme activities of C, N, P and S cycling in representative soils (0–5 and 5–15 cm) of the semiarid region of Puerto Rico as affected by management and land use. Soil organic C (OC) at 0–5 cm was higher under pasture (2–3-fold) and mango (*Mangifera indica*) trees (1.6-fold) compared to vegetable production, and similar in vegetable production (average for four soils: 15.8 g kg<sup>-1</sup> soil) and quenepas (*Melicoccus bijugatus*) trees (15.9 g kg<sup>-1</sup> soil). Soil microbial biomass C (MBC = 167–1401 mg C g<sup>-1</sup> soil) was higher in soils under trees (up to 2.4-fold) and pasture (>2.5 times at both depths) compared to vegetable production. Similar trends were found for soil MBN among the systems. Principal Component Analysis (PCA) showed differences in the soil microbial community structure under pasture and trees due to higher fungal FAME markers (i.e., 18:2ω6c, 18:1ω9c, 16:1ω5c and 18:3ω6c) compared to agricultural soils under vegetable production. Unique FAMES for soils under pasture were: 20:4ω6c, 18:1ω5c, 14:1ω5c, 11Me18:1ω7c, 15:1ω6c and i15:1. Higher number of fatty acids was extracted (51–55) from soils under pasture than in vegetable production (36–45). Several enzymatic activities (i.e., β-glucosaminidase, β-glucosidase, alkaline phosphatase and different pools of arylsulfatase) were higher (up to 4-fold) in soils under pasture, and under trees compared to the vegetables production soils. Differences found in the soil microbial community and enzymatic activities among systems have potential to be reflected in the soil functional integrity and ecosystem services, and should be considered when altering land uses to less conservative practices in the region studied.

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## 1. Introduction

While there is great interest in determining global biodiversity and the role of microorganisms in ecosystems functioning, it is important to recognize that there is little information on the soil microbial communities as affected by management and

land uses in semiarid regions, which occupy about 40% of the planet's surface (Dick-Peddie, 1991). Microbial communities are key to soil quality and functioning due to their involvement in organic matter dynamics, nutrient cycling and decomposition processes including detoxification from xenobiotics. Thus, by characterizing microbial diversity and

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composition, we may be able to better understand and manipulate ecosystem functions because the ability of an ecosystem to withstand serious disturbances may depend in part on the microbial component of the system (Nannipieri et al., 2003). Characterization of soil microbial community structure is possible by comparing fatty acids derived from the phospholipid components of the cellular membranes of microorganisms. The fatty acid methyl esters (FAME) technique by using a commercially available gas chromatograph-software system (Microbial ID, Inc. [MIDI], Newark, DE, USA) provides a fast, simple, cost effective, and reproducible method (Cavigelli et al., 1995; Ibekwe and Kennedy, 1999; Acosta-Martínez et al., 2004). Although a limitation of this method is the possible inclusion of FAMES from non-microbial material, the FAME profiles obtained were shown to be sensitive to changes in soil microbial communities as affected by management and land use similar to trends found with other methods (Schutter and Dick, 2000; Acosta-Martínez et al., 2004). Within the FAME profiles, individual FAME markers can be used to compare the relative abundance of specific microbial groups. The relative abundance of bacterial populations has been determined with the FAMES 15:0,  $\alpha$ 15:0, i15:0, i16:0,  $\alpha$ 17:0 and i17:0 (Wright, 1983; Walling et al., 1996; Zelles, 1997). Actinomycetes abundance has been determined from 10Me16:0, 10Me17:0 and 10Me18:0 (Kroppenstedt, 1992; Zelles, 1997) and the FAME marker 20:4 $\omega$ 6c has been suggested for the evaluation of protozoan abundance (Walling et al., 1996). Fungal populations have been evaluated using suggested saprophytic fungal FAMES such as 18:2 $\omega$ 6c and 18:3 $\omega$ 6c (Frostegard and Bååth, 1996) and arbuscular fungal mycorrhiza (AFM) indicators such as 18:1 $\omega$ 9c and 16:1 $\omega$ 5c (Olsson, 1999; Madan et al., 2002).

Changes in the microbial community structure are likely to be reflected in the functional integrity of the soil (Insam, 2001) because the microbial communities influence the potential of soils for enzyme (i.e., hydrolases)-mediated substrate catalysis (Kandeler et al., 1996). Important soil enzyme activities to organic matter decomposition and nutrient (C, N, P and S) transformations can be affected by soil management such as  $\beta$ -glucosidase activity, which is key in the last limiting step of cellulose degradation (C cycle) and arylsulfatase activity, important on soil organic S mineralization.  $\beta$ -Glucosaminidase activity may provide information of chitin degradation in semiarid soils as it is a key enzyme involved in the hydrolysis of N-acetyl- $\beta$ -D-glucosamine residue from the terminal non-reducing ends of chitoooligosaccharides. This hydrolysis is considered to be important in C and N cycling in soils because it participates in the processes whereby chitin is converted to amino sugars, a major source of mineralizable N in soil (Ekenler and Tabatabai, 2002). The phosphatases are crucial in organic P transformation, but are also significantly affected by soil pH, which controls P availability independent of organic matter content or levels of disturbance.

Currently, there is no information about the microbial biomass and community structure and enzyme activities as affected by management and land use in the semiarid region of Puerto Rico, a tropical island territory of the United States located in the Caribbean. More than 50% of this semiarid region is under pasture of native and improved grasses used mainly for beef production, which represent more conserva-

tive management for these semiarid soils. There are also several hundred hectares drip irrigated for export market crops from trees of avocados (*Persea americana*), mangoes (*Mangifera* spp.) and quenepas (*Melicoccus bijugatus*), which litter production and lack of tillage may also provide some benefits for soil quality and functioning. However, a considerable amount of land is intensively tilled to produce different types of vegetables during the year such as sweet peppers (*Capsicum annum*), tomatoes (*Lycopersicon esculentum*), watermelon (*Citrullus lanatus*) and/or others. The tilled vegetable systems may represent a crop rotation, which have been reported to provide positive effects on soil properties due to higher C inputs and diversity of plant residues returned to soils in comparison to continuous systems (Miller and Dick, 1995; Friedel et al., 1996; Robinson et al., 1996; Moore et al., 2000). However, tillage practices, which are intense for vegetation production, have shown to decrease soil organic C (Franzuebbers et al., 1995; Deng and Tabatabai, 1997), enzyme activities (Deng and Tabatabai, 1997; Acosta-Martínez et al., 2003), microbial biomass (Franzuebbers et al., 1994, 1995) and fungal populations (Frey et al., 1999; Pankhurst et al., 2002). Thus, we believe that soils under pasture will sustain higher microbial communities and metabolic potential compared to vegetable production that should be quantified. Previous studies have reported for other semiarid regions that native pasture showed up to 2–5-fold higher soil MBC, and higher fungal populations, when compared to agricultural systems at 0–5 cm (Acosta-Martínez et al., 2007). However, it is uncertain if differences between the soil microbial communities and enzyme activities can be characterized under pasture compared to land under trees (i.e., mango and quenepas production), and under trees (mango and quenepas) compared to vegetable production. Therefore, this study compares the microbial biomass C and N, FAME profiles of the microbial communities, and selected enzyme activities of C ( $\beta$ -glucosidase,  $\beta$ -glucosaminidase), N ( $\beta$ -glucosaminidase), P (acid phosphatase and alkaline phosphatase) and S (arylsulfatase) cycling in four representative semiarid soils under native pasture, trees (i.e., mangoes, quenepas), and vegetables production. The results of this study are expected to expand our understanding of the microbial biomass and community structure and enzyme activities involved in phosphorus, carbon, nitrogen and sulfur cycling in semiarid soils as affected by different management.

## 2. Materials and methods

### 2.1. Sites characteristics and soil sampling

The semiarid region of Puerto Rico covers 117,000 ha and is located in the southern part of the island. The annual precipitation in this region ranges from 762 to 1016 mm, and the annual ambient temperature ranges from 20 to 31 °C. Ten sites were chosen, which comprised four major soil series in the region. Each soil was under representative agricultural production (i.e., mangoes, quenepas, watermelon and vegetables) and the native pasture counterparts (Table 1).

Soil samples were collected in summer of 2005 using an auger (5 cm diameter) at 0–5 and 5–15 cm soil depths. A

Soil series classification		Land use and vegetation description	Texture (%) (0–15 cm)			pH (soil:H <sub>2</sub> O, 1:2.5)		Organic C (g kg <sup>−1</sup> )		Total N (g kg <sup>−1</sup> )	
Classification	Parent material		Sand	Silt	Clay	0–5 cm	5–15 cm	0–5 cm	5–15 cm	0–5 cm	5–15 cm
San Antón		>15 years									
Cumulic Haplustolls	Alluvial fans and flood plains formed in alluvium weathered from volcanic rock and limestone	Pasture: <i>Sporobolus indicus</i>	43	23	34	7.4	7.5	30.5a	15.6a	2.9a	1.4a
Fine-loamy, mixed, superactive, isohyperthermic		Agriculture: Mangoes ( <i>Mangifera indica</i> )	30	26	44	8.0	8.0	24.8b	14.4a	2.0a	1.2a
		Agriculture: Different vegetables (tomatoes, sweet pepper) under disk tillage	17	26	57	7.8	6.5	15.2c	15.8a	1.3b	1.3a
Jacaguas		>20 years									
Fluventic Haplustolls	Soils occur on nearly level to gently sloping flood plains close to the stream channel	Pasture: <i>Sporobolus indicus</i>	51	22	28	6.7	6.8	23.5a	16.3a	1.9a	1.4a
Loamy-skeletal, mixed, superactive isohyperthermic		Agriculture: Quenepas ( <i>Melicoccus bijugatus</i> )	44	21	35	7.7	7.6	15.9b	13.3a	1.4ab	1.1a
		Agriculture: Different vegetables (tomatoes, sweet pepper) under disk tillage	33	25	41	7.1	7.0	12.1b	11.8a	1.1b	1.1a
Pozo Blanco		>15 years									
Aridic Calciustolls	Semiarid mountain and valleys. Formed in clayey and loamy marine sediments	Pasture: Pangola grass ( <i>Digitaria eriantha</i> ) under livestock activities	37	31	33	8.2	8.2	46.5a	31.2a	3.8a	2.6a
Fine-loamy, mixed, superactive, isohyperthermic		Agriculture: Sweet pepper ( <i>Capsicum annum</i> )	34	26	39	8.7	8.7	14.1b	14.1b	1.3b	1.3b
Aguilita		>10 years									
Aridic Calciustolls	Loamy marine sediments. Formed in material weathered from soft limestone bedrock	Pasture: Kleberg bluestem grass ( <i>Dichanphium annulatum</i> ) under livestock activities	28	33	39	8.3	8.5	41.4a	24.7a	3.7a	2.4a
Coarse-loamy, carbonatic, isohyperthermic		Agriculture: Six months under watermelon ( <i>Citrulluslanatus</i> ) under moldboard plow and 6 months under grasses and livestock activities	29	24	47	8.3	8.3	22.0b	21.8a	2.1b	2.1a
Soil classification according to Beinroth et al. (2003).											

completely randomized sampling approach was used to allocate four replicates per site, except that three replicates were taken from San Antón and Jacaguas soils. For each field replicate, four locations were combined to make composite samples. The samples were kept at 4 °C until soil microbiological analysis was performed within 2 weeks of sampling and soil moisture was determined after drying at 105 °C for 48 h. A subset was air-dried for other analyses.

## 2.2. Chemical and physical analyses

Soil texture was determined on 100–400 mg of air-dried soil (<2 mm) by a laser diffraction technique using a particle size analyzer (Beckman-Coulter LS-230). The determination of soil texture using the LS-230 was significantly correlated to the pipette method (Zobeck, 2004). Soil pH was measured on the air-dried soil (sieved to <5 mm) using a glass combination electrode with a soil:water ratio of 1:2.5. Soil organic C (OC) and total N (TN) contents were determined on the air-dried soil (sieved to <180 µm) by automated dry combustion using the Vario Max-ELEMENTAR CN-analyzer (D-63452 Hanau; Germany).

Leaves from sites under trees were analyzed in a private laboratory for lignin content (Padmore, 1990), and for nitrogen (Miller et al., 1988), phosphorus (Padmore, 1990), sulfur (Blancher et al., 1965; Hoeft et al., 1973) and other nutrients such as potassium, calcium, manganese, magnesium, zinc, iron and copper (Isaac, 1990).

## 2.3. Microbial biomass C (MBC) and N (MBN)

The MBC and MBN were determined on a 15-g oven-dry equivalent field-moist soil sample (sieved to <5 mm) by the chloroform-fumigation-extraction method (Vance et al., 1987). In brief, organic C and N from the fumigated (24 h) and non-fumigated (control) soil were quantified by a CN analyzer (Shimadzu Model TOC-V/CPH-TN). The non-fumigated control values were subtracted from the fumigated values. The MBC and MBN were calculated using a  $k_{EC}$  factor of 0.45 (Wu et al., 1990) and  $k_{EN}$  factor of 0.54 (Jenkinson, 1988), respectively. Each sample had duplicate analyses and results are expressed on a moisture-free basis.

## 2.4. FAME profiles

Fatty acids were extracted from the soil samples following the MIDI (Microbial ID, Inc.) protocol as previously applied to soil analyses (Cavigelli et al., 1995; Acosta-Martínez et al., 2004). Briefly, 3-g (sieved to <5 mm) field-moist soil samples were treated according to the four steps of the MIDI protocol for biological samples: (1) saponification of fatty acids at 100 °C with 3 ml 3.75 M NaOH in aqueous methanol [methanol:water ratio = 1:1] for 30 min; (2) methylation (esterification) at 80 °C in 6 ml of 6 M HCl in aqueous methanol [1:0.85] for 10 min; (3) extraction of the FAMES with 3 ml of 1:1 [v/v] methyl-tert-butyl ether/hexane; and (4) washing of the solvent extract with 1.2% [w/v] NaOH. The FAMES were analyzed in a 6890 GC Series II (Hewlett Packard, Wilmington, DE, USA) equipped with a flame ionization detector and a fused silica capillary column (25 m × 0.2 mm) using H<sub>2</sub> (ultra

high purity) as the carrier gas. The temperature program was ramped from 170 to 250 °C at 5 °C min<sup>-1</sup>. The FAMES were identified and their relative peak areas (percentage) were determined with respect to the other FAMES in a sample using the Aerobe method of the MIDI system. The FAMES are described by the number of C atoms, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of molecules, cis isomers are indicated by c, and branched fatty acids are indicated by the prefixes i and a for iso and anteiso, respectively. Other notations are Me for methyl, OH for hydroxy and cy for cyclopropane.

## 2.5. Enzyme activities

The activities of β-glucosidase, acid phosphatase, and alkaline phosphatase were assayed using 1 g of air-dried soil (sieved to <5 mm) with their appropriate substrate and incubated for 1 h (37 °C) at their optimal pH as described by Tabatabai (1994). The activity of β-glucosaminidase was determined similarly by the method of Parham and Deng (2000). Arylsulfatase activity was determined in the field-moist soil (sieved to <5 mm) by the chloroform fumigation method described by Klose and Tabatabai (1999). This method determines arylsulfatase activity in a set of samples fumigated with chloroform for 24 h in the absence of toluene, and on the non-fumigated counterparts. The activity of the chloroform-fumigated samples is considered the total arylsulfatase activity, and the intracellular activity (enzymes from microbial cell cytoplasm) was obtained by the difference of the activity of fumigated samples and non-fumigated samples. All enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation.

## 2.6. Statistical analysis

Differences due to management and soil depth were calculated using the MIXED procedure in SAS (SAS system, 2003). The LSMEANS option was used to calculate the significant differences of the chemical, microbial and biochemical properties attributable to the agricultural production systems (vegetable, mangoes or quenepas) compared to the native system (pasture) for each soil. Principal Component Analysis (PCA) was performed for the soil FAME profiles, using the PRINCOMP procedure in SAS, to demonstrate differences in the microbial community composition of agricultural production systems compared to pasture by including most (90%) of the fatty acids extracted from the set of soils studied. PCAs were also performed for each soil with the PRINCOMP SAS procedure using the following indicator FAMES: 10Me16:0, 10Me17:0, α15:0, i15:0, α17:0, i17:0, cy19:0, 18:1ω9c, 18:2ω6c and 18:3ω6c groups. Exploratory analysis of the FAME data was performed by stepwise discriminant analysis (SDA) using the STEPDISC procedure in SAS to identify the FAMES most important to discriminate among the systems for all soils together. Canonical discriminant analysis (CDA) was performed with the CANDISC procedure in SAS using 18 FAMES identified by SDA. Pooled canonical correlations were studied to determine the association between the discriminant functions and the predictors within the system groups. The

**Table 2 – Selected properties of the leaves from mango and quenepa trees**

Properties (mg kg <sup>-1</sup> )	Mango	Quenepa
C	384.8	441.7
N	9.0	12.3
C:N	43	36
Lignin	154	344
P	0.5	0.6
K	2.6	1.2
S	1.2	1.5
Ca	60.7	49.3
Mg	1.3	7.9
Zn	28.0	58
Fe	2464	1211
Mn	435	167
Cu	9.2	9.4

first and second canonical discriminant functions ( $P < 0.001$ ) were used to plot and determine the pattern of how the systems are differentiated by FAMES.

### 3. Results

#### 3.1. Selected chemical and physical properties

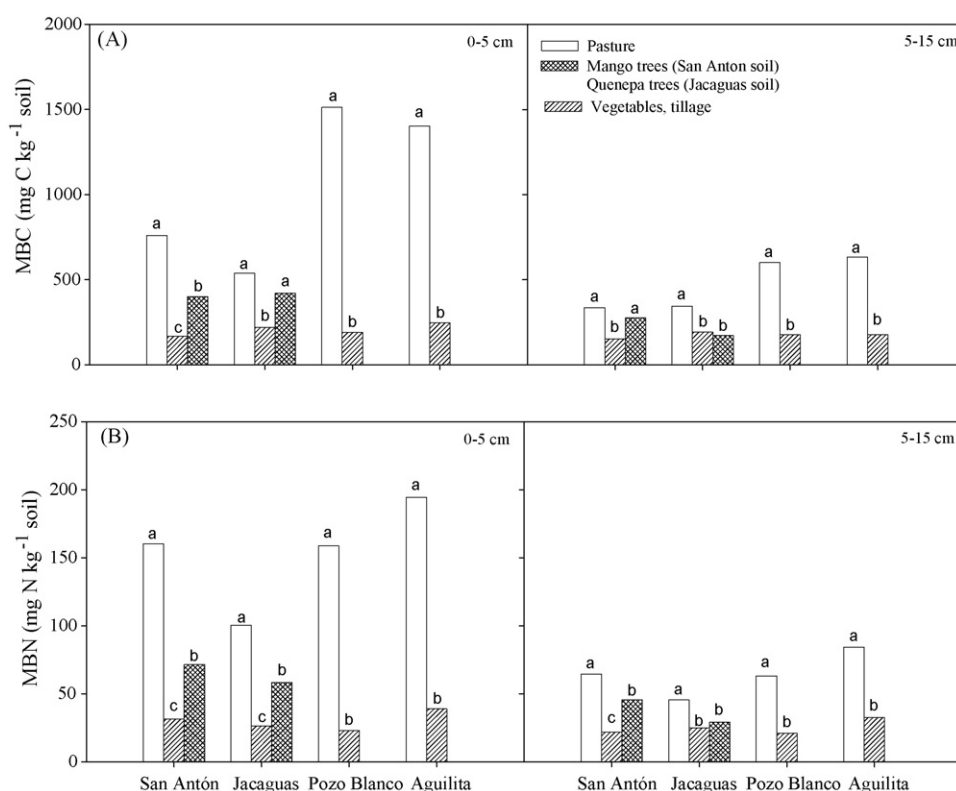
The clay content of the soils (0–15 cm) ranged from 28 to 39% in the pasture sites, and from 39 to 57% in the vegetable sites (Table 1). Soil OC was two (most soils) to three (Pozo Blanco) times higher under pasture than in the agricultural soils under

vegetable, mango or quenepa production. Differences at lower soil depth (5–15 cm) in soil OC between pasture and vegetable production were only found for the Pozo Blanco soil. Soil OC and TN were higher under mango trees than under vegetable production (San Antón soils), but similar soil OC and TN were detected under quenepa compared to vegetable production (Jacaguas soils) at 0–5 cm depth. Both soil OC and TN showed decreases with depth ( $P < 0.05$ ) under pasture and trees (quenepa and mango).

For the sites under trees, the leaves from quenepa trees contained (dry basis) higher C and N (up to 1.3 times), lignin (2 times), Mg (6 times) and Zn (2 times) than leaves from mango trees (Table 2). On the other hand, leaves from mango trees contained higher K (2 times), Ca (1.2 times), Fe (2 times) and Mn (3 times) compared to quenepa leaves.

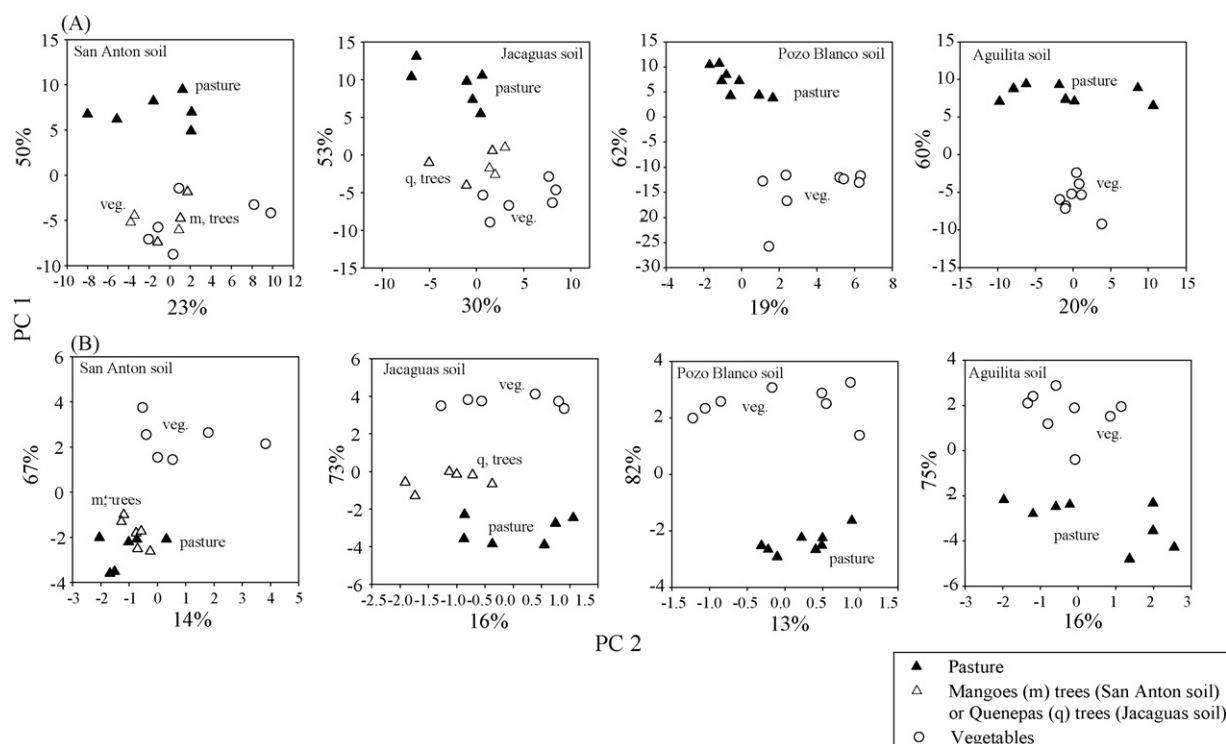
#### 3.2. Microbial biomass C (MBC) and N (MBN)

Soil MBC was 2.4 times (Jacaguas soils), 3 times (San Antón soil), or 6.6 times (Aguilita and Pozo Blanco soils) larger under pasture compared to the corresponding agricultural sites under vegetable production (Fig. 1A). Differences were also found in MBC at the 5–15 cm depth due to management or land use. Soil MBC was 2.4-fold higher under mangoes (San Antón soils) and quenepa (Jacaguas soils) trees compared to vegetables production at 0–5 cm depth. Soil MBN was higher in pasture soils compared to their agricultural counterparts at both soil depths (Fig. 1B). Generally, the soil MBN showed the following decreases within the systems studied: pasture > mango or quenepa > vegetables.



**Fig. 1 – Soil microbial biomass C (A) and microbial biomass N (B) under pasture, trees (mango and quenepa) and vegetables at 0–5 and 5–15 cm depths. Bars with different letters within a soil represent significant differences at  $P < 0.05$ .**





**Fig. 2 – Principal Component Analysis (PCA) of whole FAME profiles (A) and for 11 indicator FAMES for fungal and bacterial populations (B) in semiarid soils under pasture, trees (mango, quenepa) and vegetables at 0–5 and 5–15 cm depths. PCAs for microbial group indicators were performed using the following FAMES: 18:1 $\omega$ 9c, 18:2 $\omega$ 6c, 18:3 $\omega$ 6c; 16:1 $\omega$ 5c (fungal populations) and a15:0, i15:0, a17:0, i17:0, cy19:0, 10Me16:0, 10Me17:0 (bacterial populations).**

### 3.3. Microbial community composition

The PCAs developed for whole FAME profiles showed separation (PC1) between pasture and vegetable production systems for all soils, and there was no separation of the FAME profiles in soils under trees of mango (San Antón) or quenepa (Jacaguas soil) compared to the vegetable production counterparts (Fig. 2A). For San Antón soils, PCAs using FAMES (11) indicators for fungal and bacterial groups showed that the sites under mango trees and pasture clustered together, and there was separation of those systems from vegetables production along PC1 (Fig. 2B). For Jacaguas soils, the PCAs developed for FAMES (11) indicators for fungal and bacterial groups showed separation of pasture, quenepa trees and vegetables production.

Higher amounts of fatty acids were extracted from pasture compared to soils under vegetable production at 0–5 cm and 5–15 cm (Table 3). For example, 52 FAMES were extracted from Pozo Blanco soils under pasture compared to only 36 extracted from vegetable production soils. The FAMES generally unique to pasture soils were: 18:1 $\omega$ 5c, 10Me18:0, 15:1 $\omega$ 6c, i14:0 3OH, 20:4 $\omega$ 6c and 11Me18:1 $\omega$ 7c (data not shown). The FAMES indicators for bacterial populations a15:0 and a17:0 and actinomycetes (10Me16:0 and 10Me17:0) were higher under pasture for most of the soils studied compared to vegetable production at 0–5 cm. The fungal FAMES (18:1 $\omega$ 9c, 16:1 $\omega$ 5c, 18:2 $\omega$ 6c and 18:3 $\omega$ 6c) were higher under pasture soils and mango and quenepa trees compared to vegetable production. FAMES unique to soils under mango (14:1 $\omega$ 5c, 15:1 $\omega$ 6c

and 20:0) or quenepa (14:1 $\omega$ 5c and i16:1) trees compared to vegetable production soils were also identified (data not shown).

The stepwise discriminant analysis showed that 18 FAMES discriminated among the systems when the 4 soils were compared in a CDA plot (Fig. 3). The FAMES i16:0, 16:0, a17:0 and 18:1 $\omega$ 7c showed stronger influence on the positive side of canonical function 1 (axis 1), where all soils under pasture clustered. The FAMES i15:0, a15:0, 10Me16:0 and 18:1 $\omega$ 9c showed a strong influence on the negative side of axis 1, where all vegetable production sites were clustered and San Antón soils under mango trees. In addition, the FAMES i16:0, 10Me16:0, 16:1 $\omega$ 5c and 18:3 $\omega$ 6c showed the strongest influence on the positive side of canonical function 2 (axis 2), where Aguilita and Pozo Blanco soils under pasture and San Antón soils under mango trees were clustered. Conversely, the FAMES i15:0, a15:0, 16:0 and 10Me16:0 had the strongest influence on the negative side of axis 2, where the following systems clustered: all vegetables soils, Jacaguas and San Antón soils under pasture, and Jacaguas soils under quenepas production.

### 3.4. Enzyme activities

$\beta$ -Glucosaminidase activity was 3–5-fold higher in all soils under pasture compared to vegetable production at 0–5 cm, and the same held true for Pozo Blanco and Jacaguas soils at 5–15 cm depth (Fig. 4A). San Antón and Jacaguas soils showed

**Table 3 – FAME abundance in semiarid soils under different land use and management**

Soils	Total FAMEs extracted		Bacteria (%)			Actinomycetes (%)			Fungi (%)			
	0–5 cm	5–15 cm	i15:0	a15:0	a17:0	i17:0	10Me16:0	10Me17:0	18:2ω6c	18:3ω6c	18:1ω9c	16:1ω5c
San Antón												
Pasture	55	51	2.79a	2.81a	2.16a	1.63a	3.35a	2.63a	6.62a	2.66a	8.45a	7.12a
Mangoes trees	47	41	2.41a	2.18a	1.46b	1.90a	2.43b	1.59b	4.26b	2.32a	7.29b	7.85a
Vegetables	45	37	2.31a	0.63b	1.16b	0.63b	1.58c	0.61c	3.41c	1.91b	3.25c	3.92b
Jacaguas												
Pasture	51	48	2.68a	2.79a	2.02a	2.01a	3.75a	2.94a	5.30a	3.28a	7.08a	7.21a
Quenepas trees	42	37	2.63a	2.74a	1.23b	1.60b	3.64a	2.62a	3.41b	2.33b	6.77b	6.06b
Vegetables	41	36	2.78a	0.52b	1.09b	0.50c	1.94b	0.55b	3.56b	1.31c	3.09c	4.07c
Pozo Blanco												
Pasture	52	49	2.90a	2.48a	2.94a	2.17a	2.17a	3.26a	4.53a	2.61a	9.52a	7.35a
Vegetables	36	37	1.10b	0.36b	1.69b	0.61b	1.88b	1.12b	2.86b	1.60b	5.96b	3.15b
Aguilita												
Pasture	51	53	2.72a	2.25a	1.13a	1.25a	2.49a	2.30a	5.01a	2.98a	7.09a	6.45a
Vegetables	44	47	2.29a	0.69b	1.20a	0.51b	1.27b	1.55b	3.76b	1.85b	4.11b	4.22b

Values reported are means of four field replicates ( $n = 4$ ), except for San Antón and Jacaguas soils ( $n = 3$ ).

this trend in this enzyme activity at 0–5 cm: pasture > mangoes or quenepas > vegetable production. Similar trends were found for  $\beta$ -glucosidase activity (Fig. 4B). This soil enzyme activity was similar under pasture and quenepa in the Jacaguas soil, but higher under pasture than under mango production in San Antón soil. The activities of alkaline and acid phosphatases showed generally this trend at 0–5 cm: pasture = mangoes (San Antón soil) = quenepas (Jacaguas soil) > vegetable production (Fig. 4C and D). Significant ( $P < 0.05$ ) differences were also found between pasture and vegetable production for some of the soils at 5–15 cm.

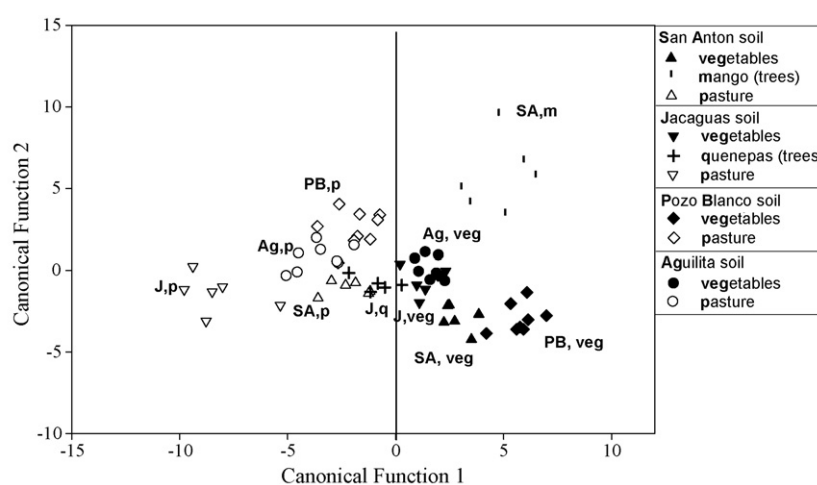
Total arylsulfatase activity, determined in chloroform-fumigated soils, showed the same trends (pasture > trees (mangoes or quenepas) > vegetable production) of the other enzyme activities as affected by the land use and management

(evaluated only at 0–5 cm depth) (Fig. 5A). Similar trends were found for the activity of arylsulfatase determined in non-fumigated field-moist soil (Fig. 5B). No significant differences were found for the released intracellular arylsulfatase activity (fumigated minus the non-fumigated soil) due to management, which represented 47% of the total arylsulfatase activity (Fig. 5C).

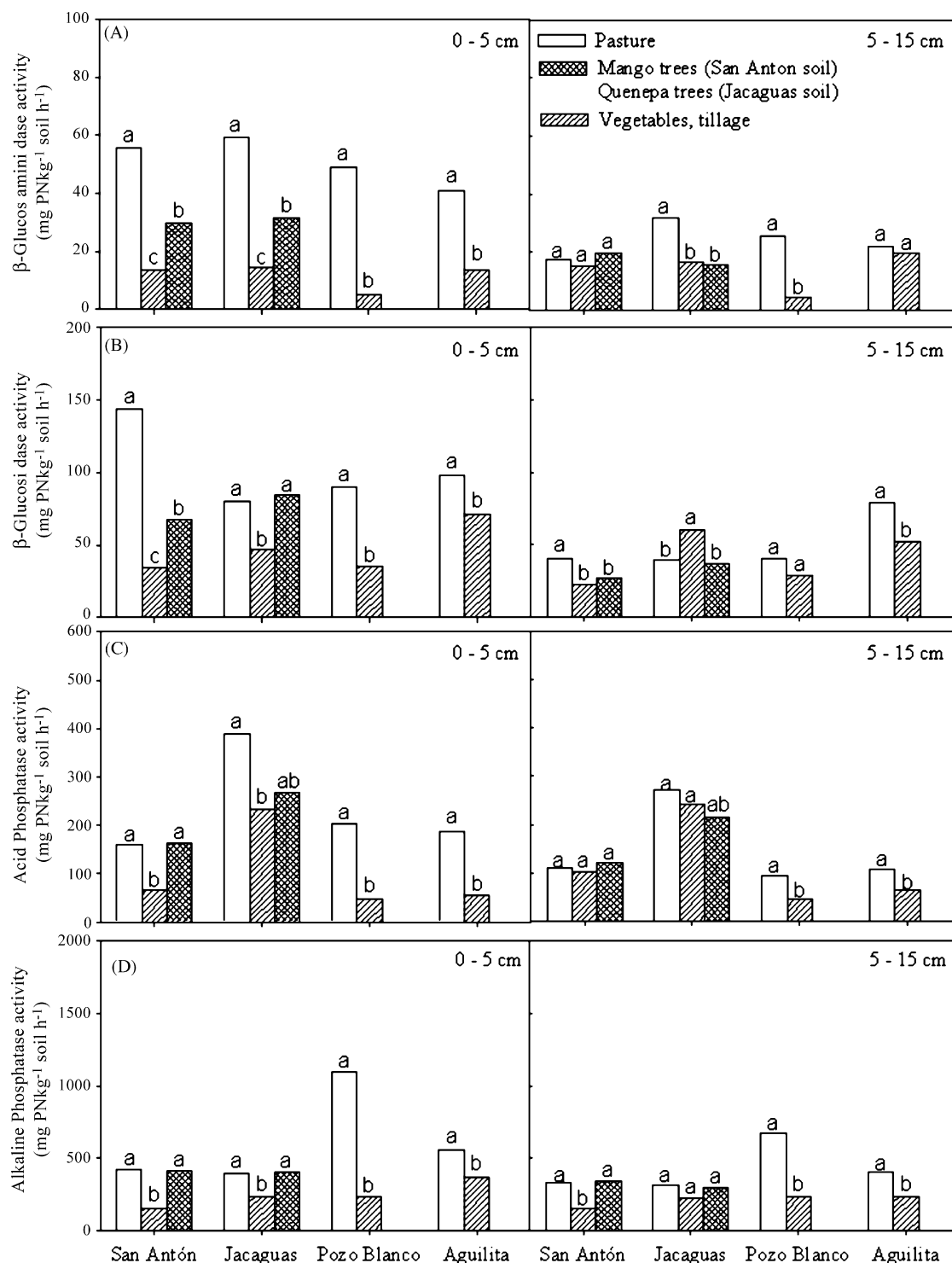
## 4. Discussion

### 4.1. Soil organic C and total N

Identification of management with positive effects in soil OC accumulation is crucial because previous studies have



**Fig. 3 – Plot from Canonical discriminant analysis (CDA) for the four soils (0–5 and 5–15 cm) studied using 18 FAMES (i13:0, i14:0, 14:0, i15:0, a15:0, 16:0N alcohol, i16:0, 16:1ω5c, 16:0, 10Me 16:0, i17:0, a17:0, 10Me17:0, 18:2ω6c, 18:1ω9c, 18:1ω7c, 16:1ω7c, 18:2ω6c) identified by stepwise discriminant analysis (SDA). Canonical functions 1 and 2 showed significant ( $P < 0.001$ ) grouping among the systems due to differences in the FAME profiles.**

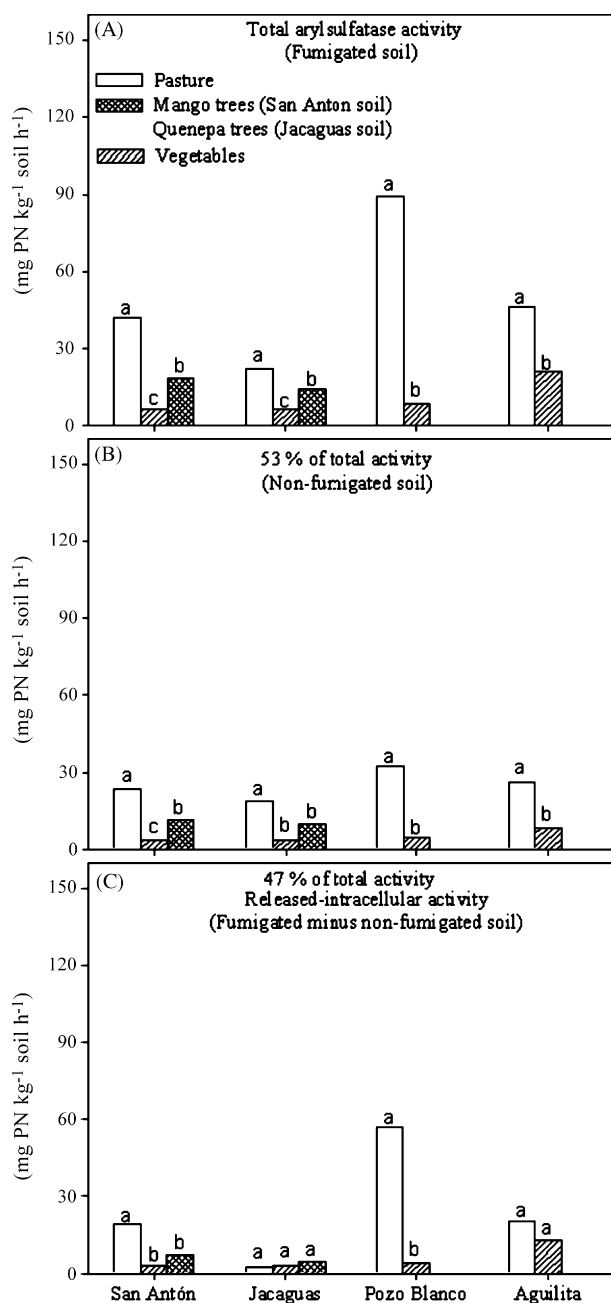


**Fig. 4 – The activities of  $\beta$ -glucosaminidase (A),  $\beta$ -glucosidase (B), acid phosphatase (C) and alkaline phosphatase (D) in soils under pasture, trees (mango and quenepa), and vegetables at 0–5 and 5–15 cm depths. Bars with different letters within a soil represent significant differences at  $P < 0.05$ .**

estimated that the loss of soil C content, such as the significant reductions of soil C found in the vegetable sites compared to pasture after >10 years, can possibly take longer in being restored (>50 years) with appropriate management (Lal et al., 1998). Our findings with agricultural semiarid soils under tilled

vegetable production compared to pasture agree with the estimations made in the United States that many soils have lost 30–50% of the C that they contained prior to cultivation (Kucharik et al., 2001). The significant reductions in soil OC content in the vegetable sites demonstrated greater oxidation





**Fig. 5 – Arylsulfatase activity of chloroform-fumigated soil (A), non-fumigated soil (B), and fumigated minus non-fumigated soils (C) under pasture, trees (mango and quenepa), and vegetables at 0–5 cm depth. Bars with different letters within a soil represent significant differences at  $P < 0.05$ .**

of soil organic matter due to intensive tillage practices compared to non-disturbed pasture soils after 10 years. In fact, vegetable sites showed higher clay content compared to the non-disturbed pasture soils due to the intensive long-term tillage operations (Bronson et al., 2004). Livestock activities, such as grazing and manure addition, typical of pastures must have increased the differences in soil OC and TN between pasture and vegetable production. Thus, conservation tillage,

livestock activities and permanent vegetation demonstrated to have positive effects in soil OC accumulation. Previous studies in other regions reported that changing cropland to perennial grassland can lead to increases in carbon sequestration, and thus, in soil aggregate stability and microbial biomass and activity (Karlen et al., 1999; Potter et al., 1999; Acosta-Martínez et al., 2004).

#### 4.2. Soil microbial biomass C and N

The size of the microbial biomass is controlled by the long-term C input into the soil (Moore et al., 2000), which explains that soils under vegetable production with lower soil OC and TN contents compared to pasture or land under mango trees showed also significantly lower (2.4–6 times depending on the soil) microbial biomass C and N. The fact that there was higher soil microbial biomass under quenepa trees compared to vegetable production, but no significant differences in soil OC content demonstrate differences in soil organic matter quality (i.e., labile pools) between these two systems. Previous studies have demonstrated the importance of litter quality and its decomposition rates (Rutigliano et al., 2004). It is possible that the similar soil OC and TN contents under quenepa trees and vegetable production may be attributed to a slower degradation of quenepa leaves compared to mangoes leaves due to higher lignin content (2-fold) and lower nutrient (i.e., Fe, Mn) contents in quenepa leaves compared to mango leaves. These findings may also be associated to soil N limitations as sites under quenepa trees generally require less fertilization than mango trees. Thus, significant differences in organic matter content may take even longer to be detected between the soils under quenepa and vegetable production because microbial biomass has generally a faster turnover than soil organic matter (Sparling, 1997). According to this study, mango or quenepa production can conserve soil labile organic matter pools similar to the native undisturbed (pasture) system, and much higher than under vegetable production in this semiarid region. Interestingly, all soils showed similar levels of MBC and MBN in vegetable production, which may be due to their similar clay content and edaphic properties (i.e., all soils are Mollisols).

#### 4.3. Microbial communities

The distinct grouping of the FAME profiles under pasture compared to vegetable production soils suggested a dominant influence of permanent surface cover, livestock activities and lack of tillage on the microbial community structure that were in agreement with the observed higher soil OC, MBC and MBN under pasture. The different microbial biomass and community composition of pasture soils were due to higher abundance of FAMES for bacterial (i.e., i17:0 and a15:0), actinomycetes (10Me16:0) and fungal populations (i.e., 18:3 $\omega$ 6c and 16:1 $\omega$ 5c) in comparison to agricultural soils under vegetable production at 0–5 cm. In addition, higher fatty acids were extracted from pasture including unique FAMES in this system such as an indicator for actinomycetes (10Me18:0) and protozoan (20:4 $\omega$ 6c) at 0–5 and 5–15 cm. The protozoan FAME trends suggest that a better environment was available in pasture soils compared to the other systems at the time of

sampling (i.e., summer) as these microorganisms are unable to withstand long periods of low soil moisture and high temperature (Mayzlish and Steinberger, 2004). Despite being less predominant, ongoing studies using direct count techniques for the same soils have revealed differences in protozoan diversity under the different land use and management evaluated, which demonstrate the importance of using different approaches to better characterize soil microbial diversity and abundance (Acosta-Mercado and Lynn, 2004). In general, our findings agree with previous studies where the soil microbial community structure of pasture was reported to be significantly different compared to agricultural counterparts, but the differences reported can depend on the soil, vegetation and/or agricultural management (Acosta-Martínez et al., 2004; Martens et al., 2004). For example, while we found unique FAMES associated to pasture sites, others have reported no differences in the number of fatty acids extracted from prairie and agricultural counterparts, but the FAME abundance have differed among these two systems (McKinley et al., 2005; Acosta-Martínez et al., 2007).

Although soils under pasture and under trees represent undisturbed systems due to lack of tillage, PCAs for whole FAME profiles showed separation of soils under pasture and under trees (mango, quenepa), but no separation of soils under trees and vegetable production, which may demonstrate that the surface cover and substrates (organic matter, C and N) from pasture rhizosphere played more significant impacts on the whole FAME profiles of the microbial communities compared to the rhizosphere under trees. However, PCAs using indicator FAMES for microbial groups separated the soils under trees (mango or quenepa) from vegetable production due to higher abundance of the fungal FAMES 18:3 $\omega$ 6c in soils under trees. In addition, the dominance of arbuscular mycorrhizal fungal (AMF) indicators 18:1 $\omega$ 9c (Madan et al., 2002) and 16:1 $\omega$ 5 (Olsson, 1999) in pasture and land under trees compared to vegetable sites is of ecological significance due to the several benefits of mycorrhiza on soil quality and functioning (nutrient cycling, soil structure, etc.). In agreement with our findings, Drijber et al. (2000) reported higher 16:1 $\omega$ 5c in grassland compared to cropland and under no-tilled systems compared to tilled counterparts. Thus, fungal populations were enhanced under trees and pasture due to the lack of hyphae disturbance under no-tilled conditions, and to the benefits of more permanent plant residues or vegetation cover compared to tilled vegetable production (Frey et al., 1999; Acosta-Martínez et al., 2004; Kennedy and Schillinger, 2006). In contrast, agricultural soils, like those under vegetable production in our study, where residues are buried with conventional tillage, have shown lower microbial biomass due to presumably higher predominance of bacterial populations (Coleman et al., 1983; Calderón et al., 2001; Kennedy and Schillinger, 2006). Our CDA plot performed for all soils together confirmed that management played a more significant role in the microbial communities regardless of the soil type because all soils under tilled vegetable production clustered together. Differences in the microbial communities found in pasture and land under trees compared to vegetable production can provide indications of differences in other soil properties. Previous

studies reported that tilled systems with less biomass returned to the soil have shown higher rate of substrate degradation resulting in a decrease in several soil properties including soil organic matter, soil structure, fungal biomass and nitrification (Doran, 1982; Elliott, 1986; Karlen et al., 1994).

#### 4.4. Enzyme activities

The differences found in soil microbial community structure as affected by vegetation and tillage management modified the potential of soil enzyme-mediated substrate catalysis (Kandeler et al., 1996). The enzyme activities were correlated to the response of the microbial biomass ( $r > 0.67$ ;  $P < 0.05$  for all soils). The higher microbial biomass under pasture, mangoes or quenepas trees compared to vegetable production was in agreement with higher activities of  $\beta$ -glucosidase and  $\beta$ -glucosaminidase, which are involved in the release of carbohydrates in soil. Carbohydrates represent an important (labile) component of soil organic matter and provide the major substrate source for soil microorganisms. Martens et al. (2004) reported that there should be three to five times greater amounts of carbohydrates in surface horizons of more conservative management systems that provide plant biomass, vegetation cover, and lack of tillage such as pasture and forest systems, and this may apply for the vegetative litter produced under mango and quenepa trees. Although, we generally found higher activities of the glycosidases ( $\beta$ -glucosidase and  $\beta$ -glucosaminidase) under pasture compared to mango or quenepas (except for Jacaguas soils), there were generally similar activities of the phosphatases under these systems perhaps because the soil pH was not affected by the management of these systems. These enzyme activities are known to be more significantly affected by changes in soil pH than in organic matter content. At least, leaves from mangoes and quenepas trees showed similar P levels (0.5–0.6 mg kg<sup>-1</sup>).

The higher enzyme activities in soils under pasture or trees of mango or quenepas can be due to the more active microbial biomass (intracellular enzymes) or to the microbial biomass producing more enzymes that then became stabilized in soil organic matter (extracellular enzymes). However, it could also be due to both situations (Klose and Tabatabai, 1999; Acosta-Martínez et al., 2004). Because enzyme assays available cannot distinguish between different sources of the phosphatases or glycosidases in soil, we used the method suggested by Klose and Tabatabai (1999) to distinguish between total and intracellular (microbial) arylsulfatase activity. Similar to previously reported, we found differences in the response of the different pools of arylsulfatase activity to management depending on the soil (Klose et al., 1999). For example, Jacaguas and Aguilita soils under pasture showed higher microbial biomass and fungal populations compared to vegetable production, but there was no significant difference of intracellular arylsulfatase activity under pasture and vegetable production. Klose et al. (1999) explained that the lack of statistically significant correlations between the intracellular arylsulfatase activity and microbial biomass may indicate that not all components of the microbial community are sources of arylsulfatase activity in soils.

## 5. Conclusion

Results demonstrated 30–50% C content reduction in semiarid soils due to intensive tillage cultivation for vegetable production compared to undisturbed pastures, which resulted in a community structure with lower fungal populations and lower enzyme activities after >10 years. The beneficial effects of tree litter biomass return and lack of soil disturbance on the microbial community structure and biochemical functioning for these semiarid soils were demonstrated. The effects of litter quality on soil C accumulation were also demonstrated. Although soil microbial biomass was higher under quenepa trees compared to vegetable production, the soil organic C was similar between those systems, which suggest the importance of modifying management for land under trees experiencing similar trends in other semiarid soils to encourage litter degradation and nutrients return to the soil.

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